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Tonsillar Syphilis: an Unusual Site of Infection Detected by *Treponema pallidum* PCR

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With the reemergence of syphilis, it is important that both clinical and public health practitioners recognize the various clinical manifestations of this disease (formerly known as "the great imitator") and become familiar with the newer diagnostic tests. Here we report the first case of tonsillar syphilis diagnosed by PCR.

CASE REPORT

In October 2014, a 58-year-old homosexual male in a long-term exclusive relationship with a man presented to the Gay Men's Health Clinic at Our Own Health Centre in Winnipeg, Manitoba, Canada, with a 2-week history of sore throat and left otalgia. He confirmed that he still had only one partner. On examination, his left tonsil was unusually smooth, red, and quite enlarged. His left tonsillar lymph node was tender and was significantly enlarged to 2.5 to 3 cm in diameter. The result of otoscopy of the left ear was normal, and he was given a presumptive diagnosis of viral tonsillitis. A throat swab for beta-hemolytic streptococci was negative by culture. He declined sexually transmitted infection (STI) screening at his first visit.

He returned a week later feeling anxious because he remembered that he had had an extrarelationship contact involving oral sex with another man at a party about 8 weeks earlier (that was approximately 5 weeks prior to the onset of otalgia). He stated he had never received anal sex. On examination, there was now a thin film of white slough on the surface of his left tonsil with no other lesions or patches found in his mouth. He was treated with penicillin V (600 mg every 12 h [q12h]) for 12 days, pending return of laboratory investigations ordered on the basis of the revised history provided and consent for STI screening. A throat swab for culture and a urine specimen for a nucleic acid amplification test (NAAT) were both negative for *Neisseria gonorrhoeae*. A direct fluorescent antibody assay (DFA) of a throat swab and a NAAT of urine specimens were both negative for *Chlamydia trachomatis*. A second throat swab taken for routine culture was again negative for beta-hemolytic streptococci. A serum sample was sent for syphilis, hepatitis B virus (HBV), and human immunodeficiency virus (HIV) serology at the Cadham Provincial Public Health Laboratory in Winnipeg, Manitoba, Canada. The patient had previously tested negative for syphilis serology by the use of a treponema-specific antibody a year prior to the current visit. Because of his clinical presentation and the history that he provided, as well as the ongoing syphilis outbreak happening among men who have sex with men (MSM) in Winnipeg, a throat swab (Dacron in viral transport medium at 4°C) was also sent to the National Microbiology Laboratory (NML) in Winnipeg, Canada, for syphilis PCR testing. When the tonsil was swabbed for testing, a shallow erosion was revealed with superficial bleeding. He subsequently tested negative for both HBV (by a surface antigen test) and HIV (by an HIV-1/2 antibody plus HIV p24 antigen combo assay). Syphilis serology results were as follows: *Treponema pallidum* antibody positive by both chemiluminescent microparticle immunoassay (CMIA) and *Treponema pallidum* particle agglutination (TP-PA) (4+) along with a Venereal Disease Research Laboratory (VDRL) reactive result of a 1-in-16 dilution (a titer of 16). Subsequently, a syphilis PCR tested positive for *Treponema pallidum* DNA using three different gene targets (*polA*, *bmp*, and *tpp47*) as previously described (1). PCR and sequencing of the 23S rRNA gene were also done as part of the reflex testing algorithm at the NML, which revealed an A2058G mutation in the strain, characteristic of macrolide resistance (2, 3).

A week later, the patient came back for a follow-up visit. On examination, he had no fever, malaise, organomegaly, nuchal rigidity, eye or ear symptoms, skin rashes, or genital, pubic, or scrotal chancres. There was also no cervical, axillary, or inguinal lymphadenopathy or any other manifestation of secondary syphilis detected on this visit (or on the first or second visit). He was given 2.4 million units of benzathine penicillin G intramuscularly. The serum sample collected on the third visit had the same results as the previous draw (VDRL titer of 16). Both partners of the patient had been notified by public health practitioners, who had been informed of the positive syphilis results and of the need for contact tracing and epidemiological treatment. Another serum specimen collected 4 weeks after treatment showed a VDRL titer of 4 (4-fold drop), and, finally, the serum specimen collected 12 weeks after treatment showed a

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VDRL titer of 1 (16-fold drop, exceeding the treatment goal for primary syphilis).

Syphilis has without a doubt been a reemerging infectious disease in the past decade in developed countries such as the United States and Canada, with a number of the newly reported outbreaks happening, especially among MSM (4–6). Syphilis is a sexually transmitted disease caused by the bacterium *Treponema pallidum* subspecies *pallidum* (7). Syphilis has been known for centuries to have so many clinical manifestations as an infectious disease that it has historically been called "the great imitator." With the discovery of penicillin in early 20th century, there was a drastic reduction in number of syphilis cases. Primary, secondary, and early latent syphilis cases are normally treated with a single dose of penicillin G, and the bacterium has never developed any resistance to this antibiotic, probably due to the lack of transposable elements carrying resistance genes (8, 9). However, due to a global change in sexual practices throughout 20th century, the incidence of syphilis has increased. With the adoption of safer sexual practices during the HIV pandemic, the incidence of syphilis in the MSM community diminished to insignificant numbers. However, since the middle 1990s and early 2000s, after antiretroviral therapy (ART) was introduced and became known to control HIV and prevent AIDS, there has been a significant rise in the number of newly acquired syphilis cases in both the United States (6) and Canada (10), with the latter experiencing a 481% increase in incidence. This is believed to be due to a number of factors, including the acceptability of the risk of treatable STIs to MSM, the advent of on-line "hook-ups," the misconception that ART can prevent other STIs, including syphilis, and the introduction of a serosorting approach, especially by HIV-positive MSM. The majority of the outbreaks have occurred among MSM, most of whom acquired the disease through unsafe sexual practices (8).

The natural history of syphilis usually begins with a highly variable incubation time of 3 weeks to 3 months (the so-called "incubating syphilis" period). Classical presentation of primary syphilis may include a nontender, usually indurated skin nodule (or more than one in HIV-infected patients) known as a syphilitic chancre(s) at the site of inoculation, typically being present in the genital area, including the glans or shaft of the penis, the perineal or perianal area, inside the vagina or anal canal, or in the oral cavity (7). There have been only a few reports of primary syphilis with tonsillar chancre as the sole manifestation, especially in the context of high titers of anti-treponema-specific antibodies and VDRL titers as low as 16. In one recently published case report, the patient also had several other oral and perioral lesions as well as skin rashes, with a concomitant VDRL titer of 256 (11), and the diagnosis was established based only on clinical manifestations and serological test results. In a more recent case report from Italy, Drago et al. (12) reported a case similar to that of our patient, though the throat swab from their patient grew *Staphylococcus aureus* and they performed a tonsillar biopsy followed by DFA using rabbit polyclonal antiserum to establish the diagnosis. However, DFA using polyclonal antiserum is not specific enough to rule out the presence of common oral treponemes such as *Treponema denticola*. It is also important to note the invasive nature of biopsy, which is a significant limiting factor in the current practice. Furthermore, the difficulty of obtaining positive-control slides for DFA is another deterrent to this diagnostic approach. Our report is distinguished from the previous ones in two ways: (i) the sole clinical manifestation was the unilateral tonsillar involvement, and (ii) aside from robust serological diagnosis, PCR was employed as a noninvasive and reliable diagnostic modality, as this test is not affected by the presence of common oral treponemes with the current test setup and the three specific gene targets used (1).

Molecular methods such as PCR with subsequent nucleotide sequencing provide an excellent opportunity for genotyping and molecular epidemiological investigations (13, 14) as well as for confirming the site or potentially the portal of infection. In genotyping, *T. pallidum tpr* genes are amplified by PCR followed by digestion with restriction enzymes to obtain a profile for restriction fragment length polymorphism (RFLP) analysis. Alternatively, the acidic repeat protein gene (*arp*) is amplified and sequenced. Molecular analysis also allows detection of point mutations (A2058G and/or A2059G) in the 23S rRNA gene that confers macrolide resistance (2, 3). If the *T. pallidum* strain is susceptible, azithromycin may be considered a useful alternative to penicillin G with a single 2-g oral dose for treatment of syphilis or for postexposure prophylaxis, especially in those without concomitant HIV infection but with a severe penicillin allergy (15). Another application of molecular analysis is to distinguish endemic treponematoses (yaws, pinta, and endemic syphilis) from venereal syphilis as we have reported recently (16) since lesions may appear in both and venereal syphilis is usually prevalent in countries where endemic treponematoses are also common. It is important that, based on our experience, up to 20% of syphilis cases that test positive by PCR also test negative by a very sensitive CMIA. This fact is of the utmost public health importance, since, during the course of primary syphilis with lesions that are teeming with spirochetes, individuals are highly infectious; PCR is thus important as an adjunct to serology in early diagnosis of infection and subsequent contact notification and epidemiological treatment.

Conclusion.

Oropharyngeal/tonsillar chancres of primary syphilis have been previously reported in the literature but to the best of our knowledge have never been tested by PCR. In the context of the concurrent and emerging syphilis outbreaks in industrialized countries along with concomitant changing sexual practices, health care providers should be alerted to unusual manifestations of primary syphilis and become cognizant of the diagnostic modalities available in their jurisdiction to establish fast and reliable diagnosis. It is also important to bear in mind that positive syphilis PCR results, like positive serological test results, are notifiable to public health departments by both laboratories and health care providers, thus enabling them to have a better grasp of the transmission dynamics and, ultimately, better control of syphilis, "the great imitator."

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